Item 3. The non-technical abstract

Information for Item 3. The non-technical abstract is provided below.

One approach to improving the treatment of cancer is to deliver most of the anti-cancer agent directly to the tumor, thus concentrating the effect on the tumor and avoiding toxicity to normal tissues. Some bacteria have the interesting property of accumulating preferentially within tumors following intravenous (IV) or direct tumor injection in animals, reaching very high numbers in the tumor compared to normal tissues. Thus, bacteria could be used to deliver anti-cancer agents to tumors, if they could be administered without causing the serious consequences of infection such as damage to normal organs and, in severe cases, septic shock and death.

We have modified a type of *Salmonella* bacteria by taking out two of their genes. These weakened bacteria, called VNP20009, can be given safely at high doses to mice with implanted tumors, by the IV route or by direct injection into tumor. These bacteria maintain their property of preferentially accumulating within the tumors. Based on this information, we have started human clinical trials of VNP20009. In one of the clinical studies, we have shown that VNP20009 can be injected directly into tumors, and so far, only minimal side effects are encountered. VNP20009 persists in the tumor for at least 2 weeks in most patients. VNP20009 are also not shed from the body in stool or urine, which indicates that they are unlikely to spread to health care workers or other people.

We have now modified VNP20009 further to carry a bacterial gene that produces an enzyme called cytosine deaminase (CD), in order to increase the effects of the bacteria against the tumor. The bacteria are called TAPET-CD or VNP20029. In terms of toxicity and ability to accumulate preferentially in tumor, TAPET-CD behaves very similarly to VNP20009 in animal models. The CD has a specific purpose, which is to convert a relatively non-toxic pro-drug called 5-fluorocytosine (5-FC) to the anti-cancer agent 5-fluorouracil (5-FU). When the pro-drug 5-FC is given (in mice, into the abdominal cavity where it is absorbed into the blood), it circulates within the body. It then gets converted to the more toxic drug 5-FU in the tumor, but not in other parts of the body, because of the preferential accumulation of TAPET-CD (and therefore CD) in the tumor. The 5-FU that is produced locally within the tumor can then kill tumor cells. We found that the combination of the TAPET-CD bacteria and 5-FC was safe in animal models and produced tumor growth inhibition, and in some cases, caused shrinkage of the animal tumors.

Based on the information in the animal models, a clinical study is proposed to test the combination of TAPET-CD and 5-FC in patients with advanced cancer that have exhausted all effective treatment options. The bacteria will be injected directly into a tumor. After 3 days, the drug 5-FC will be given by mouth 3 times per day for 14 days. The cycle of bacterial injection and 5-FC treatment is repeated every 28 days if the tumor shows signs of stabilization or shrinkage, and if other tumors in the body are not growing.